# ALLOXAN DIABETES
# ITS PRODUCTION AND MECHANISM*

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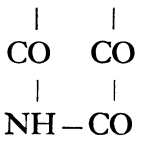
ALLOXAN DIABETES is a new type of experimental diabetes. It is a chemical diabetes caused by treatment with an organic compound, which is related to physiological body substances. It can be produced in the intact animal and in many species. Its signs and symptoms are identical in many respects with those of human diabetes. It is the purpose of this paper to describe the production and the patho-physiology of this diabetes and to compare its mechanism with that of the various other types of experimental diabetes.

Alloxan has been known for more than 100 years, since Woehler produced it by oxidation of uric acid. It has the following structure:



is a colorless powder, melting at 256 degrees centigrade, and is easily soluble in water and alcohol. It decomposes on hydrolysis into urea and mesoxalic acid. Alloxan reduces to dialuric acid with which it acts as an oxidation-reduction system, and its structure is found as a constituent part of the flavine molecule. It has been found to produce profound disturbances in sulfur metabolism, to enhance the endogenous metabolism of liver suspensions, and to be a capillary poison. In spite of this, little evidence exists so far that alloxan plays a role in physiological processes. Uric acid metabolism, so far as is known, does not seem to involve alloxan even as an intermediary product.

The discovery of the diabetogenic action of alloxan is accidental

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as many other great discoveries in science. It is the great merit of the English pathologist Shaw Dunn, whose recent death put an untimely end to a distinguished career, to have observed that alloxan had a specific necrosing action on the cells of the islets of Langerhans. Working on the crush syndrome and the effect of ureides on the kidney, he and his co-workers made this "side observation" and called attention to its importance. Six years earlier, in 1937, Jacobs[1] had found that the injection of alloxan into rabbits resulted in an initial hyperglycemia which was soon succeeded by a severe hypoglycemia, lasting up to eight hours or longer, and terminating with death of the animals unless glucose was administered. All his experiments, however, were acute, and no histological studies were done. Dunn, Sheehan and McLetchie,[2] noticed again this fluctuation of blood sugar in alloxan-treated animals, recognized its relationship to the accompanying anatomical islet cell damage and though their rabbits had all died in a few days, suggested that a sustained diabetes should be the final result of alloxan poisoning.

Their report stimulated work by other investigators, and in only a few months, alloxan diabetes was demonstrated in various animal species. In July, 1943 the production of alloxan diabetes in dogs, and of transitory hyperglycemia and glycosuria in rabbits was announced from our laboratory.[3] Bailey and Bailey reported on alloxan diabetes in rabbits,[4] and Shaw Dunn and his group[5] produced alloxan diabetes in the rat. In the meantime, confirmations and further contributions have been made by several workers in this country and abroad.[6-9]

*The diabetogenic doses:*

In our laboratory the diabetogenic effect of alloxan has been studied in the dog, the rat, the cat, the rabbit, the rhesus monkey, the pigeon, and the guinea pig.[10-14]

Alloxan diabetes can be produced with one single injection of alloxan. We use freshly prepared unneutralized 5 per cent solution, and give it intravenously or intraperitoneally because of its decided acidity. Neutralization inactivates alloxan, and it decomposes on standing. Scattered small doses and subcutaneous injections have been used also with success.

We define the diabetogenic dose as the amount of alloxan which in eighty per cent of the animals of a given species will produce sus-

tained hyperglycemia and necrosis of the pancreatic islet cells, but which will not cause observable damage to other organs.

TABLE I

*Diabetogenic Doses of Alloxan in Various Species*

| | | |
|---|---|---|
| Rat | 200-300 | mg/kg i. p. |
| Rabbit | 100-200 | mg/kg i. v. |
| Cat | 150 | mg/kg i. v. |
| Monkey | 100-150 | mg/kg i. v. |
| Dog (dalmatian hound) | 50-100 | mg/kg i. v. |
| Pigeon | 125-200 | mg/kg i. v. |

Table I shows the doses as we have found them for various species. It must be mentioned, however, that the response of the cat is very erratic and that of the pigeon develops other metabolic changes frequently, which will be discussed later.

The greatest sensitivity and smallest required dose are found in dogs. Next in sensitivity are monkeys, pigeons and cats; rats and rabbits require the largest dose. The carbohydrate metabolism of the dalmatian hound and the pigeon, which, like man, do not convert uric acid into allantoin, responds in the same way as the animals do which produce allantoin from uric acid.

If a dose larger than the diabetogenic dose is given, severe kidney damage is produced also, and anuria and nitrogen retention develop, and the animals succumb in a few days in a uremic-diabetic syndrome. Still larger doses are fatal within a few hours, probably because of the effect of alloxan on the circulatory system; there is evidence of acute pulmonary edema. The margin between the fatal, the uremic and the diabetogenic dose is the widest in rabbit, rat and dog, smaller in the monkey, and smallest in pigeon, cat and guinea pig. In the latter, no exact diabetogenic dose could be established as yet, since degenerative lesions in the pancreas were found only in those animals which died within 24 hours after injection of alloxan.

*The clinical course:*

Typical diabetes mellitus will develop about 24 to 28 hours after the injection of a diabetogenic dose of alloxan. The classical signs and symptoms of hyperglycemia, glycosuria, polyuria, and polydipsia will

be present, and very frequently polyphagia and loss of weight. In some species early ketonuria can be observed.

During the first day after the injection, careful observation and supervision are necessary in rabbits and monkeys, lest they die in hypoglycemic convulsions. Frequent administration of glucose may be required. Dogs, rats, and pigeons show less marked initial fluctuations of the blood sugar, and may survive without protection by glucose.

Dogs with alloxan diabetes appear well for about two to three weeks. Their blood sugar remains at a level between 200 and 300 mg. per 100 cc., and their glycosuria may reach four to seven per cent. They eat with great appetite and lose little weight during this period. Glucose tolerance tests show a typical diabetic curve, and insulin sensitivity tests prove that the animals are sensitive to insulin from the very beginning of their diabetes.

Under insulin treatment, the dogs remain in good condition for many months. About one unit protamine zinc insulin per kilo per day is necessary to prevent loss of weight, to maintain normal fasting blood sugar, and to keep the urine free or almost free of sugar. If the dogs remain untreated for more than two or three weeks, they stop eating, lose weight rapidly, appear weak, listless and drowsy, and are very susceptible to infections. This condition is accompanied by the development of hyperlipemia and fatty degeneration of the liver, a phenomenon which may be of importance for the problem of lipocaic, since—as will be shown later—the alpha cells remain intact in alloxan diabetes. The diabetic symptoms very seldom show spontaneous improvement, and usually persist until death occurs in complete emanciation at six to seven weeks.

Monkeys have a very high blood sugar and marked glycosuria following the initial hypoglycemia; ketonuria is present very early. The monkeys look sick and depressed, become emaciated rapidly, and go into acidosis. This is remarkable since monkeys who have been pancreatectomized do not always develop severe diabetes.

Rats, too, show a rapidly progressing diabetes and die in ketosis and acidosis in six to nine days if untreated. Out of a series of 27 rats, which we injected with alloxan, only four did not develop diabetes. All the others showed marked glycosuria and hyperglycemia within 24 to 48 hours. The blood sugar reached values of 300 to 500 mgm. per cent. On the third or fourth day, ketonuria appeared in those animals whose

blood sugar was higher than 300 mgm. per cent. Most impressive was the polyuria: the daily urinary output often exceeded 10 per cent of the body weight. The weight loss averaged 30 per cent in six days. Insulin treatment controls this alloxan diabetes.

The alloxan diabetes of the rabbit also responds to insulin therapy, but its course in the untreated rabbit may take two entirely different forms.

Some animals react, as do dogs, rats and monkeys, and lose weight rapidly, become seriously emaciated, and die within a week. Their blood sugar level is between 400 and 500 mg. per cent, and there is a 5 to 10 per cent glycosuria.

Another group of rabbits respond with similar levels of blood and urine glucose, but after an initial slight weight loss seem to recuperate and regain their weight, appearing in excellent health. Such animals survive without insulin treatment for months, despite the severe diabetes and are apparently capable of compensating for the loss of glucose in the urine by increasing their food intake.

A few rabbits may give the impression of clinical cure of the diabetic condition, the blood sugar returns to normal, the urine is sugar free, but a persistently decreased glucose tolerance test remains to indicate the diabetes.

Of great interest is the development of systemic changes usually considered secondary to diabetes. The great susceptibility to infections has been mentioned before. Of greater importance is the development of cataract which has been observed first by Bailey[15] and of retinitis which was reported by Lewis. It seems, however, that other factors besides the diabetes may contribute to these eye complications. They are not found in all alloxan diabetic animals, and have not yet been observed in our laboratory. So far we have no explanation for this surprising fact.

Pigeons which after total pancreatectomy usually do not develop hyperglycemia and survive indefinitely, respond to alloxan injection with an elevation of the blood sugar to 400 or 500 mg. per 100 cc. They appear sleepy, weak and drowsy, and sit with their eyes closed, making no attempt to move or fly, and die in this stupor. Death, however, may not be due to the diabetes alone; many of the animals develop a very interesting second condition, which has been known for a long time as visceral gout. The blood uric acid content shows a marked

increase to 100 times the initial level and at autopsy, the pericardium and other serous membranes are found to be covered with sodium urate crystals; the kidneys are infiltrated with the same material.

*The histological changes:*

Only a few remarks on the histological changes after alloxan treatment, which will be dealt with in detail in the following paper by G. Gomori. The pancreatic changes are practically identical in all species mentioned and are characterized by a selective necrosis of the beta cells, as has been shown first in our laboratory, not of the whole islet system. Whereas the beta cells undergo rapid degeneration and disappear finally completely, the alpha cells remain undamaged, at least retain their normal staining property. The beta cell necrosis proceeds without any sign of inflammatory reaction. In dogs the pancreatic ducts usually show a characteristic vacuolization. Of extra-pancreatic changes, the glycogen deposition in the kidney, accompanying pronounced glycosuria, and the necrosis of the convoluted tubules are of importance, the latter occurring only if amounts larger than the diabetogenic doses have been given. The fatty infiltration of the liver, which is most marked in the dog, has been mentioned before. How rapidly the islet cell necrosing action of alloxan starts and how quickly alloxan is made innocuous or removed from the blood stream could be demonstrated recently in the following, not yet published, experiment. We deprived part of the pancreas of its blood supply by means of a clamp, which was released five minutes after the injection of alloxan. Biopsies were taken 24 hours later from both parts of the pancreas and showed that the islet cells appeared essentially normal where the circulation had been interrupted for five minutes only, and that the rest of the pancreas which had had normal blood supply had undergone the typical beta cell degeneration.

*The mechanism:*

If the degeneration of the beta cells leads to deficient insulin production and thus to diabetes, bioassays of the pancreatic tissue should reveal changes in the insulin content. Assays were carried out on the pancreas of three dogs with alloxan diabetes of a duration of 18, 30, and 60 days. In all three, the insulin content of the pancreatic tissue was markedly decreased. Whereas the pancreas of a normal dog con-

tains about 2 to 3 units of insulin per gram, only ¼ of this amount was recovered from the alloxan-treated organs. This seems to be conclusive evidence that alloxan diabetes is a true pancreatic diabetes and is the result of insulin deficiency. This experiment has found confirmation recently in the work of Ridout, Ham and Wrenshall.[16]

How does alloxan exert its effect upon the islet cells?

Let us recall the early blood sugar fluctuation after alloxan: first a short-lasting hyperglycemia, then a transitory hypoglycemia which in the rabbit frequently requires treatment with glucose, and finally the sustained diabetic hyperglycemia. What is the cause and the significance of these three phases? We shall discuss first the hyperglycemia as a whole, then the initial hyperglycemic phase alone, and finally the hypoglycemia. It is known from the surgical diabetes after partial pancreatectomy, as well as from anterior pituitary diabetes that the initial hyperglycemia precedes the islet cell degeneration and that diabetes will not develop if the hyperglycemia is prevented by starvation, insulin treatment or phlorhizin injection.[17] Here the hyperglycemia is a causal factor of the ensuing diabetes.

In alloxan diabetes such protection is not possible. The diabetogenic process will take its course regardless of whether the initial hyperglycemia is prevented or not. Alloxan affects the islets cells directly.

In a series of experiments which can only be summarized here, we have been able to show that treatment with phlorhizin as well as treatment with insulin will prevent the initial alloxan hyperglycemia, but in spite of the normal blood sugar level the islet cell degeneration will develop. Two dogs were treated for a period of ten days with phlorhizin; on the eighth day they received a diabetogenic dose of alloxan. Diabetes developed as if no phlorhizin had been given. Another dog was given insulin together with and four days following the injection of alloxan. As soon as insulin treatment was discontinued, a marked hyperglycemia and glycosuria became evident. Diabetes had developed as if no insulin had been given. Insulin treatment was renewed after a few days. The same dose was sufficient for diabetic control; there was no change in the sensitivity to insulin. When after a week insulin was stopped, the diabetes became evident again, and a biopsy of the pancreas revealed islet cell degeneration to the same extent as in animals not treated with insulin at all.

Now the early hyperglycemic phase: If insulin is injected together

with alloxan—either as a mixture into the same vein, or separately into different veins—no initial hyperglycemia will develop in the rabbit. The hypoglycemic phase, however, is of the usual severity, and diabetes results as if alloxan alone had been given. The fact that insulin prevents the initial hyperglycemia argues against the possibility that the mechanism is one of insulin inhibition. This possibility was ruled out further when we found that insulin was not inactivated by alloxan in vitro. It seems, therefore, likely that the initial hyperglycemia is not due to lack of insulin, but to mobilization of extra glucose. Such glycogenolysis might be produced by the liver under the influence of epinephrine.[18] As a matter of fact, Young and his associates[9] were able to reproduce the initial alloxan blood sugar curve by the injection of adrenalin and insulin into normal rabbits. We subjected this hypothesis to experimental test by the injection of alloxan into functionally or anatomically adrenalectomized rabbits. The adrenals were extirpated surgically or the medulla was destroyed by intramedullary injection of formalin. A diabetogenic dose of alloxan was given immediately after the removal of the second adrenal or two days after the formalin injection. In no instance did any marked initial hyperglycemia develop. All animals went into severe hypoglycemia and in the few surviving animals diabetes developed. It can be concluded from this experiment that adrenal stimulation of gluconeogenesis is involved in the production of the initial alloxan hyperglycemia. This conclusion is supported by the evidence of histological changes in the adrenal medulla immediately after alloxan injection given by Hard and Carr.[8]

The second phase of the blood sugar reaction to alloxan, the hypoglycemia, was originally interpreted as a result of an insulin-like effect of alloxan itself. Were this true, the blood sugar of depancreatized animals should fall in response to alloxan. We gave diabetogenic doses of alloxan to depancreatized dogs and also to rabbits which previously had been rendered diabetic by alloxan. No lowering of the blood level could be noted in either group. The presence of a normally functioning pancreas, therefore, seems to be necessary for the occurrence of the hypoglycemic reaction, and it would appear that alloxan changes glucose metabolism through its effect on the pancreatic islet cells. This conclusion is further substantiated as well by the work of Corkill, Fantl and Nelson,[7] who found that alloxan did not influence the blood sugar level of the eviscerated cat, and by Ridout and his co-workers

who worked on depancreatized dogs.[16]

If the hypoglycemia is brought about by the effect of alloxan upon the pancreas, it may be caused either by islet cell stimulation or by the release of stored insulin from the degenerating cells. The first view, put forth by Dunn and his co-workers,[2, 5] suggests that increased islet cell activity and overproduction of insulin may overstrain the cells and cause their subsequent failure and degeneration. The experimental evidence, however, seems to favor the second possibility, and we believe with Young and his co-workers[9] that the hypoglycemia is the first sign, rather than the cause, of the developing necrosis. We base our belief on the following evidence:

1. Beta cell degeneration precedes the hypoglycemia. In histological studies on rabbits and dogs we have found that beginning degeneration of the beta cells is demonstrable as early as one hour after injection with alloxan. It precedes the hypoglycemia which usually develops only after four to five hours.

2. It is impossible to separate the hypoglycemic effect from the diabetogenic action. Whenever hypoglycemia develops, diabetes follows if the animal survives.

*The specificity of alloxan:*

A few words must be said about the remarkable specificity of alloxan as a diabetogenic agent. Dunn and his co-workers[2] had found that not only alloxan but also a quinoline compound (styryl-quinoline No. 90) produced islet cell necrosis in rabbits. They tested several other compounds as oxalic acid, uranium, quanidine and uric acid, and found that none of them had a diabetogenic or islet cell necrosing effect. Earlier Jacobs[1, 19] had found that none of a series of more than sixty chemical compounds produced the fluctuation of the blood sugar level which is characteristic for early response to alloxan. No alloxan-like action was found in a series of compounds tested by Thorogood.[20] We have tested three groups of substances: compounds which are chemically related to alloxan, such as dialuric acid, the reduction product of alloxan, and alloxantin, which is formed by interaction of alloxan and dialuric acid; oxidizing agents, which were tested on the assumption that alloxan may owe its effect to its oxidizing property, and quinoline and cinchophen as relatives of styryl-quinoline, which is not available on the market. These substances were injected in various doses in dogs

and the blood sugar level as well as the histological appearance of the pancreas were followed. None of them exhibited any diabetogenic activity though several of them proved to be very toxic. The specificity of the diabetogenic action of alloxan, therefore, remains unexplained. It seems, however, unlikely that its oxidizing property is the diabetogenic factor.

*Comparison with other forms of experimental diabetes:*

In a comparison of alloxan diabetes with other forms of experimental diabetes it must be pointed out once more that the diabetes of alloxan poisoning is the result of *direct* damage to the beta cells. This chemical diabetes thus differs significantly from the surgical diabetes of partial pancreatectomy and from the endocrine diabetes of treatment with anterior pituitary extract (APE diabetes)[21,22] though all three have in common the degeneration of the beta cells. This difference can be summarized as follows:

1. Both surgical and APE diabetes are considered to be due to overwork of the beta cells since they can be prevented by starvation or by insulin in the early stages of the disease. Alloxan diabetes is the result of direct action upon the islet cells and cannot be prevented either by starvation or by insulin.

2. The pituitary extract has to be administered in increasing doses over a period of several days, while alloxan is effective in a single dose.

3. Some species, as for instance, rats, can be made diabetic by APE only after extensive resection of the pancreas, while alloxan is effective in the intact animal.

4. APE diabetes in its early stage is insulin-resistant while alloxan diabetes is not.

5. The histologic changes in APE diabetes are mitoses, degranulation and vacuolization of the beta cells with subsequent atrophy and hyaline changes. In alloxan diabetes the changes are those of an irreversible acute degeneration, without vacuolization or fibrosis.

It must be emphasized further that alloxan proved to be diabetogenic even in those species in which total pancreatectomy is not always followed by severe diabetes.

What the significance of alloxan diabetes may be for the pathogenesis of human diabetes must be left for further investigation. Whatever the outcome may be, it can be stated already that we have found

in the diabetogenic action of alloxan not only new means to study disturbed carbohydrate metabolism but also a previously unknown mechanism which causes beta cell degeneration and diabetes through the toxic effect of an organic compound related to protein metabolism.

*Therapeutic implications:*

Finally, attention should be called to a clinical implication. Brunschwig[23,24] has suggested that the islet cell-necrosing property of alloxan might be of use in the treatment of hyperinsulism and islet cell tumors. He gave alloxan to a few patients with inoperable carcinoma and to one patient with metastatic islet cell tumor. The clinical results which we observed with him were not very striking, and the histologic changes in the islet cells were surprisingly slight. The observations, however, are too limited to permit conclusions as to the advisability and effectivity of such treatment. The fact that many animal species have been found sensitive to the islet cell-necrosing action of alloxan makes it likely that man will be no exception. In any case, the remarkable—and almost unique—fact of the specific action of a chemical compound on a highly differentiated specific cell system deserves greatest interest and thorough examination.

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## *Editor's Note*

A related paper by Dr. George Gomori, Department of Medicine, The University of Chicago, entitled: "The Histology of the Normal and Diseased Pancreas," which was presented before the New York Diabetes Association, September 28, 1944, will appear in the February issue of the Bulletin together with a discussion of these two papers by Dr. Paul Klemperer, Pathologist, The Mt. Sinai Hospital, New York.